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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#7G3479/FAP#7H5523 - Myclobutanil on Apples and Grapes - Evaluation of Analytical Method and Residue Data (Accession Numbers 266026 through 266032)

RCB Nos.: 1954 through 1956

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The Rohm & Haas Company is proposing temporary tolerances for residues of the fungicide alpha-butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile and its metabolites containing both the chlorophenyl and triazole rings in or on the following:

Apples:	Wet pomace	1.0 ppm
	Dry pomace	5.0 ppm
Grapes:	Wet pomace	1.0 ppm
	Dry pomace	5.0 ppm
	Raisins	5.0 ppm
	Raisin waste	12.5 ppm
Meat and meat byproducts (except liver)		0.04 ppm
Liver (cattle, goats, hogs, horses or sheep)		0.5 ppm
Milk		0.1 ppm
Eggs		0.04 ppm

Temporary tolerances of 0.5 ppm have already been established for residues of this fungicide on fresh market apples and grapes in conjunction with PP#4G3149. These tolerances expire February 28, 1988. The purpose of the present petition is to drop the "fresh market only" restriction so that treated apples and grapes may be processed into juice, pomaces, raisins, etc. We note that the Pesticide Chemical News Guide lists established temporary tolerances for apple and grape pomaces plus raisins. This is apparently an error since such tolerances were not requested prior to the current petition.

Other names for myclobutanil include the company code RH-3866, Rally™, and Systhane™.

Permanent tolerances for residues of myclobutanil have been requested on the same commodities in PP#7F3476/FAP#7H5524. Review of the latter will commence upon our completion of this temporary tolerance request. During our review of this petition we have at times referred to the "Summary and Discussion: RH-3866 Residue Chemistry" (EPA Accession No. 266105) in PP#7F3476. Where pertinent we have mentioned information/data from that Summary in this review.

Conclusions

1a. The 60DF product has lower individual application rates than the wettable powder, but higher doses per season for both apples and grapes. Therefore, considerably more applications per season would be permitted for the DF product. The petitioner should reexamine the labels to determine if all the rates (individual and seasonal) are truly as intended. The 60DF label should also include an "oz ai per acre" rate for individual treatments of apples.

1b. More details should be given on the labels in regard to spray volumes for apples. Directions are needed for both dilute sprays (i.e., to runoff) and concentrate sprays.

1c. The "fresh market only" restrictions on the labels are contradictory to the request for byproduct tolerances and should be deleted. Revised labels should be more legible than those in the present submission. For example, the decimal points in the copy of the 60DF label are not visible so that one could mistake 24 oz for 2.4 oz.

2a. The metabolism of myclobutanil in fruits has been adequately delineated. The major metabolites are the alcohol RH-9090 (formed by hydroxylation of the #3 carbon on the butyl chain) and its glucoside. Small amounts (1 to 3% of whole fruit residue) of the ketone RH-9089 are also present. The proposed tolerance expression with metabolites containing both the chlorophenyl and triazole rings is appropriate.

2b. For the purpose of this experimental use only, the metabolism of RH-3866 in animals is adequately understood. The residue of concern is the parent compound only.

3a. Method 310-84-13 can serve as the enforcement method for temporary tolerances for residues of RH-3866 in animal commodities.

3b. Method 310-84-27 is adequate for enforcement of temporary tolerances in plants. This method determines RH-3866, RH-9089 (after reduction to RH-9090), and RH-9090 (free plus conjugated).

4a. The established temporary tolerances of 0.5 ppm for myclobutanil and its metabolites on fresh apples and grapes are adequate for the proposed use.

4b. Although the proposed tolerances of 1 ppm and 5 ppm for wet and dry grape pomace, respectively, are reasonable, it is current practice to set only one pomace tolerance covering both byproducts. A revised Section F should be submitted listing a tolerance for "grape pomace" at 5 ppm. The 1 ppm wet grape pomace tolerance should be deleted.

4c. The 5 ppm food additive tolerance for raisins is adequate. However, the raisin waste tolerance should be changed to 15 ppm to avoid fractional tolerances greater than 1 ppm.

4d. As with grapes, the 1 ppm feed additive tolerance for wet apple pomace should be deleted. Also, the 5 ppm dry apple pomace tolerance should be changed to "apple pomace".

5. Most of the proposed tolerances for residues of RH-3866 plus its metabolites in animal commodities are higher than necessary. The following tolerances should be proposed in a revised Section F under a separate heading specifying residues of myclobutanil only (see Conclusion 2b):

Meat, fat and meat byproducts (except liver) of cattle, goats, hogs, horses and sheep	0.04 ppm

Liver of cattle, goats, hogs, horses and sheep	0.2 ppm
Milk	0.02 ppm
Meat, fat and meat byproducts of poultry	0.01 ppm
Eggs	0.01 ppm

Recommendations

For the reasons cited above in Conclusions 1a, 1b, 1c, 4b, 4c, 4d, and 5 we recommend against the proposed temporary tolerances. Revised labels and Section F addressing these deficiencies are needed.

Method trials will be requested in the near future for both the total method (310-84-27) on plants and Method 310-84-13 on animal commodities. These trials do not need to be completed prior to approval of temporary tolerances.

Registration Division should ensure that the inert ingredients in Rally 60DF are approved for use on growing crops.

The petitioner should also be informed of the following additional requirements for permanent tolerances for myclobutanil on apples and grapes.

1. Although a final decision is dependent upon our review of metabolism studies in PP#7F3476, validated analytical methods will probably be necessary for the metabolites RH-9090, RH-294, and the hydroxylactone in animal tissues, milk, and eggs. A method for RH-9089 may also be required.

2. Recovery data for RH-9090 glucoside in apples and grapes at levels down to at least 0.05 ppm. Several other fortifications in the range of 0.1 to 1.0 ppm should also be tested. At this time the method for free RH-9090 is at best only marginally acceptable (8 out of 17 spiked samples in processing studies had <70% recovery). The results with the glucoside will help determine whether the method needs to be modified to improve recoveries of RH-9090. The upcoming method trial in our Beltsville laboratory will also influence our decision regarding this issue.

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3. A method for the ketone RH-9089 in apples and grapes will not be required for permanent tolerances on these crops. It is the petitioner's option as to whether the borohydride reduction should be kept in the analytical method for plants. The possibility remains that RH-9089 will be a residue of concern in animals as noted above in 1.

4. Additional residue trials for grapes (CA and NY) and apples (major production areas) reflecting the maximum application rates (per spray and per season) for both the 40W and 60DF formulations. Data for aerial applications are necessary if this mode of application is to be included on the labels.

5. Although they will probably not be needed for the uses on apples and grapes, conventional cattle and poultry feeding studies using nonradiolabeled RH-3966 may be required if future uses of myclobutanil result in higher dietary burdens for livestock.

6. Data to show the behavior or recovery of RH-3866 through the multiresidue procedures employed by the Food and Drug Administration.

Detailed Considerations

Manufacturing Process and Formulation

The production of technical RH-3866 is discussed in the Confidential Appendix to the January 9, 1985 R. Loranger review of PP#4G3149. The impurities of the technical are also listed there. Based on their chemical nature and the application rate of myclobutanil, the contaminants are not expected to create residue problems.

The formulations to be examined in the proposed experimental program are Rally 40W and Rally 60DF. The 40W formulation is apparently the same as RH-3866 40WP, a 40% active wettable powder whose composition is given in our review of PP#4G3149. The inert ingredients are approved for use on crops under 40 CFR 180.1001. We have not received information on the inerts in the 60DF product. It is RD's responsibility to ensure that the inert ingredients of Rally 60DF are cleared for use on growing crops.

Proposed Use

The dosages for both Rally 40W and Rally 60DF per application and per season are summarized in the following table.

Apples	40W	60DF
oz ai per 100 gal	0.8-1.0	0.48-0.72
oz ai per acre	2.8-4.0	Not given
max lb ai per season	2.0	3.0

Grapes	40W	60DF
oz ai per acre	0.8-2.0	0.48-1.44
max **oz** ai per season	9.6	14.4

It can be seen that the DF product has lower individual application rates but higher doses per season. Therefore, considerably more applications would be allowed per season for the DF formulation. We request that the petitioner reexamine the labels to determine if all the rates are truly as intended. The 60DF label should also include an "oz per acre" rate for individual treatments of apples.

The labels specify use of airblast or hydraulic spray equipment. Grape applications start at prebloom and continue at 14- to 21-day intervals or as needed. Apple treatments begin at green tip and may be repeated at 7- to 10-day intervals. A 14-day preharvest interval is imposed for each crop. In addition, grazing and feeding of treated cover crops in apple orchards are prohibited.

More details should be given on the label in regard to spray volumes for apples. Directions are needed for both dilute sprays (i.e., to runoff) and concentrate sprays.

Finally, we note that both labels still specify treating only fruit intended for the fresh market. This limitation is contradictory to the tolerance requests for pomace and raisins and should therefore be removed from the labels. The revised labels should be more legible than those in the present submission. For example, the decimal points in the copy of the 60DF label are not visible so that the 2.4 oz could be mistakenly read as 24 oz.

Nature of Residue

Plants

Our January 9, 1985 review of PP#4G3149 discusses metabolism studies using wheat plants (field and greenhouse) as well as wheat and grape seedlings grown in aqueous solutions of RH-3866. Below we shall discuss additional studies which employed apple trees and grape vines.

The report entitled "The Metabolism of RH-3866 in Apples" (Technical Report #310-84-31) (dated December 18, 1984) was submitted previously in PP#4G3149 (EPA Accession No. 073599) but not reviewed. This study entailed the application of either uniform phenyl ^{14}C-labeled or ^{14}C-triazole (3 and 5 positions) RH-3866 to one section of a semi-dwarf McIntosh apple tree. The location of this experiment and whether it was conducted indoors or outdoors are not indicated. However, based on the ^{14}C residue decline study conducted at the same time (see Residue Data below), this study was run at the Rohm & Haas Spring House Farm in Pennsylvania. Forty-eight milligrams of labeled material in 300 mL water [redacted] were applied to each tree to runoff using a handheld compressed air sprayer. This is claimed to be equivalent to 240 g ai/ha (0.21 lb ai/A) in 1500 L water per hectare (160 gal/A). Ten applications were made at 1- to 2-week intervals with harvest occurring 14 days after the final spray.

The apples were quartered and passed through a juicer attachment of a Hobart food chopper to produce juice and pomace. The wet pomace was frozen and ground with dry ice. Juice and homogenized pomace were stored frozen until analysis. The juice was radioassayed directly by liquid scintillation counting (LSC), whereas pomace was combusted to $^{14}CO_2$.

Juice was neutralized with sodium bicarbonate and extracted three times with chloroform and then butanol. The layers were radioassayed with the chloroform layer also analyzed directly by silica gel thin layer chromatography (TLC). The butanol was evaporated and the residue refluxed with methanolic HCl before partitioning with chloroform. The latter was analyzed by TLC. These silica gel plates were subjected to radioautography and the active zones scraped and quantitated by LSC.

The workup of pomace involved soxhlet extraction with methanol. The solvent was evaporated from the extract and the residue dissolved in water before characterizing it in the same manner as juice.

The total activities calculated as ppm RH-3866 are summarized below for the two labels.

Fraction	ppm RH-3866 Phenyl ^{14}C	Triazole ^{14}C
Whole fruit	0.48	0.32
Juice	0.15	0.12
Pomace	1.00	0.66

The whole fruit residues were calculated from the activities observed in the juice and pomace. Working backwards we calculate that the juice comprised 61 percent (phenyl label) or 63 percent (triazole) of the apple on a weight basis. Taking into account the relative weights of the two fractions, we calculate that most of the residue (81% phenyl; 76% triazole) was in the pomace even though it comprises < 40 percent of the weight of the apple.

The solvent partitioning of the radioactivity was similar for the two labels. For juice 52 to 54 percent went into chloroform, 8 to 13 percent into butanol, and 34 to 44 percent remained in the aqueous solution. The methanol soxhlet extraction removed 90 percent of the residue from pomace. This solubilized activity distributed 74 percent into chloroform, 22 to 25 percent into butanol, and 2 to 4 percent stayed in the water.

The chloroform layers from juice and pomace contained the same compounds - parent RH-3866, the alcohol RH-9090, and the ketone RH-9089 (see Attachment for structures). However, the juice contained a much higher proportion of the alcohol. The chloroform-soluble residue in juice consisted of 38 to 44 percent RH3866, 2 percent RH-9089, and 46 to 51 percent RH-9090. The chloroform layer from pomace had 72 to 74 percent parent fungicide, 1 to 2 percent RH-9089, and only 10 percent alcohol.

The hydrolyses of the butanol layers gave similar results for both labels and fruit fractions: 83 to 94 percent of the activity partitioned into chloroform after the acidic methanol treatment. By TLC 80 to 100 percent of this released activity was shown to be the alcohol RH-9090. The remainder was parent compound (up to 7%) or the ketone RH-9089 (8%). It is assumed that this acid treatment hydrolyzed the glucoside of RH-9090 based on the petitioner's experience with a wheat metabolism study. We note that the grape study (see later in this section) confirmed the presence of the RH-9090 glucoside by both TLC and enzyme (glucosidase) treatment.

Actual copies of the TLC autoradiograms were not submitted. However, computer printouts were provided listing the disintegrations per minute (dpm) of various extracts and the zones of each thin layer plate.

The overall distribution of the residue on a whole fruit basis is as follows: 49 percent RH-3866, 2 to 3 percent RH-9089, 12 percent free RH-9090, 21 to 24 percent conjugated RH-9090, and 14 to 16 percent others (unextracted, unidentified).

We conclude that an adequate study has been conducted to determine the metabolism of myclobutanil on apples. The major metabolic pathway is hydroxylation of the #3 carbon on the butyl chain with subsequent conjugation (probably to glucose). About half of the terminal residue was parent fungicide and one-third the alcohol RH-9090, about two-thirds of which was conjugated.

The "Metabolism of ^{14}C RH-3866 in Field Treated Grapes" (Technical Report #310-84-31) was also submitted in Accession No. 073599 (PP#4G3149). Grape vines at the Rohm & Haas Newton Farm (Pennsylvania), covered with bird netting, were treated five times at weekly intervals in August 1984 with either phenyl or triazole-labeled RH-3866 (38 mg ai in 300 mL water). The material was applied to both sides of the vine with a handheld compressed air sprayer. This rate is claimed to be equivalent to 0.045 lb ai/A. Foliage and two bunches of grapes were taken after each application. Seven days after the final application all remaining fruit was harvested.

Grapes and foliage from each treatment were ground with dry ice and stored in a freezer. Portions of grapes were combusted to determine total radioactivity.

The harvest grapes (final sample) were ground in a Hobart juicer and then placed in a nylon straining bag in a polyethylene wine press. After pressing for several hours, about 4.4 kg of juice were obtained from both the phenyl and triazole-labeled grapes. The weights of wet pomace for the corresponding labels were 1.41 kg and 1.12 kg. This means the percent juice by weight was 76 percent for the phenyl-labeled grapes and 80 percent for the triazole label. Upon drying overnight at ca. 75 °C, the amounts of pomace obtained were 0.49 and 0.42 kg. This corresponds to drydown factors of 2.7 to 2.9. The dry pomace was combusted for measurement of activity, while juice was directly assayed using LSC.

Sample workup for juice entailed filtering through glass wool, neutralization with sodium bicarbonate, and partitioning with chloroform (3X) followed by butanol (4X). Both pomace and foliage were extracted overnight with methanol using a soxhlet apparatus. Water was added to each extract and the solution partitioned with hexane followed by chloroform (3X) and, in the case of foliage, by butanol also. Organic extracts were concentrated under reduced pressure and radioassayed by LSC.

All chloroform extracts were examined by silica gel TLC followed by autoradiography. The radioactive bands were scraped off and counted. In the case of juice and pomace, the radioactive material was eluted from the silica and injected into a gas chromatograph (GC) for additional confirmation of identity.

To determine if conjugates were present, portions of the aqueous layers from juice and pomace were extracted with chloroform (to remove parent and nonconjugated metabolites) and then treated with a mixture of alpha- and beta-glucosidase. The solution was then extracted with chloroform and the latter examined by TLC. The radioactive spots from the juice sample were further studied by GC with electron capture detection.

The butanol extract from foliage was examined by TLC including preparative plates. Two fractions were isolated and compared by additional TLC to the standards for parent RH-3866, RH-9090, and RH-9090 glucoside.

The total activity in grapes increased erratically with the number of applications. Residues for the phenyl and triazole-labeled materials were 0.047 to 0.38 ppm (calculated as RH-3866) and 0.014 to 0.31 ppm, respectively. The harvest grapes are claimed to have 0.32 and 0.24 ppm, although we calculate values of 0.26 and 0.21 ppm based on the juice/pomace residues and the relative weights of the two fractions. The actual total activities of the various grape byproducts are summarized below.

	Total ppm ^{14}C RH-3866	
Fraction	Phenyl	Triazole
Juice	0.042	0.034
Wet pomace	0.97	0.91
Dry pomace	2.81	2.43

It appears that the wet pomace values are based on the ppm in dry pomace with a correction for the change in weight due to water.

Although the grapes were about 80 percent juice, the pomace contains about 88 percent of the whole fruit residue.

The radioactivity in juice distributed as follows into the chloroform, butanol, and final aqueous layers. For the phenyl-labeled RH-3866, the relative amounts were 64:29:7. For the triazole label the distribution was 46:23:32. The chloroform layers contained myclobutanil, the alcohol RH-9090, and the ketone RH-9089. The copy of one thin-layer plate shows faint bands at the R_f values corresponding to parent and RH-9090. The identities were also confirmed by GC retention times. Enzyme (glucosidase) treatment of the aqueous fraction (after chloroform extraction) released 65 percent of the phenyl activity and 30 percent of the triazole activity into chloroform. TLC of the latter revealed the presence of RH-9090 indicating that the RH-9090 glucoside occurs in juice.

Pomace activity was found to favor chloroform when compared to the juice results. Only 11 to 13 percent of the pomace residue was not extracted by methanol. The chloroform layer contained 81 percent (mostly RH-3866) with only 2 percent and 4 to 6 percent in hexane and aqueous layers, respectively. As with juice, a large portion (60 to 73%) of the aqueous radioactivity could be released into chloroform as free RH-9090 after glucosidase incubation.

The foliage residue was found to go primarily into chloroform (38 to 39%) and butanol (51 to 52%) with 7 to 8 percent unextracted. The butanol is claimed to contain RH-3866 and RH-9090 glucoside. Our inspection of the thin layer plate reveals a spot for RH-9089 (ketone) also. The chloroform TLC shows parent compound and free RH-9090.

The quantitation of metabolites involved direct counting of the TLC zones for all the chloroform layers and the butanol fraction from foliage. RH-9090 glucoside was quantified by determining the amount of radioactivity going into chloroform following glucosidase treatment.

The relative extent of metabolism in the different plant parts can be seen in the following table.

	Percent of Residue			
Fraction	Parent	RH-9089	RH-9090	RH-9090 Gluc.
Juice	26-33	3-4	14-23	17-24
Pomace	71-72	1	6-7	3
Foliage	47-49	2-4	11-12	16-17

Juice clearly contains the highest proportion of RH-9090 (hydroxylated RH-3866) and its glucoside.

The overall distribution of the terminal residue on a whole grape basis was almost identical for the phenyl and triazole labels: 66 percent RH-3866, 1 percent RH-9089, 7 to 9 percent RH-9090, 5 to 6 percent RH-9090 glucoside, ca. 10 percent not extracted, ca. 10 percent not identified.

With the above studies the petitioner has adequately delineated the metabolism of myclobutanil in fruits. The major metabolites are the alcohol RH-9090 and its glucoside. Small amounts (1 to 3% of whole fruit residue) of the ketone RH-9089 are also present. (See the Attachment for structures of these compounds.) Method 310-84-27 is claimed to be able to measure all these compounds, although validation data have not been provided for RH-9090 glucoside and RH-9089. The proposed tolerance expression with metabolites containing both the chlorophenyl and triazole rings is appropriate.

The fruit metabolic pathway is the same as that observed in wheat. However, in wheat germ, conjugates of alanine are also found due to soil degradation of RH-3866 and uptake of triazole and/or its conjugates into the wheat plant.

Animals

Some information has been provided in the present petition on the nature of the residue of myclobutanil in cow milk and urine (Technical Report No. 31H-86-19; EPA Accession No. 266027). This work is a continuation of the cow study for which an interim report (#310-84-12) was filed in PP#4G3149. As noted in our January 9, 1985 review of that petition, five daily oral doses of 207 to 212 mg of ^{14}C-phenyl- or ^{14}C-triazole-labeled RH-3866 were administered to a lactating dairy cow. With feed consumption of 20.8 kg/day the doses were equivalent to about 10 ppm in the diet. Milk was sampled twice daily with excreta collected once a day. The cows were sacrificed about 24 hours after the final dose. The total radioactivity was then measured in various tissues, milk, and excreta.

Since the weights of feces and urine were not determined, it is not possible to determine the proportion of the administered ^{14}C that was excreted. For the phenyl- and triazole-labeled RH-3866 the percentages of the total dose appearing in milk were 0.4 and 0.25 percent, respectively. In both cases milk residues peaked by the afternoon of day 2. Milk activity throughout the dosing period ranged from 0.024 to 0.043 ppm (as RH-3866) for the phenyl-labeled material and 0.017 to 0.034 ppm for triazole label. Tissue residues were also somewhat higher from the phenyl RH-3866: muscles, 0.011 to 0.014 ppm; fats, 0.024 to 0.036 ppm; liver, 0.42 ppm; and kidney 0.11 ppm. The corresponding values from triazole-labeled myclobutanil were < 0.0085 ppm, < 0.011 to 0.012 ppm, 0.24 ppm, and 0.059 ppm.

The interim report also included analyses of milk and tissues for the parent compound RH-3866. All but one fat sample (0.005 ppm) contained no detectable (< 0.005 ppm) RH-3866. We thus concluded previously (R. Loranger, January 1, 1985) that most of the residue in milk and tissues did not consist of RH-3866 *per se*.

The additional data now provided by the petitioner identify some of the metabolites in milk and urine. The characterization of urinary metabolites involved TLC and reverse-phase HPLC with collection of fractions for GC/MS. Identified metabolites include the alcohol RH-9090, the glycol RH-294, and the hydroxy-lactone (see Attachment for structures). One metabolite, in which the cyano function has been converted to a methyl ester, is thought to perhaps be an artifact from methylation during sample preparation.

Of greater concern is the nature of the residue in milk. Samples of the latter were homogenized, centrifuged, and then frozen to solidify separated fat ("fat pad"). After removal of the fat the supernatant was decanted off the precipitated pellet ("milk solids"). Two volumes of acetonitrile (ACN) were added to the supernatant and placed in the freezer for 2 to 6 hours. Most of the ACN was then decanted and the remaining solvent plus "precipitated proteins" centrifuged. The pellet was isolated and dried in a hood. The ACN supernatant was reduced in a rotary evaporator and five volumes of isopropanol added to precipitate "lactose." The latter was then separated from "soluble whey" by filtration. The whey solution was concentrated, placed on a C-18 Sep-Pak, washed with water, and eluted with methanol. The eluant was mixed with water and partitioned with hexane. The latter was concentrated and added to the fat pad while the aqueous layer was analyzed by reverse-phase HPLC.

From ca. 275 g of milk, the above procedure yielded about 37 g milk solids (13 to 14% of initial milk weight), 8 to 11 g fat (3 to 4%), 1.8 to 2.6 g precipitated proteins (0.6 to 1.0%), and 6.2 to 12.7 g lactose (2.2 to 4.6%). The recovery of total radioactivity was 93 to 98 percent with the following distribution: 13 to 18 percent milk solids, 2 to 4 percent fat pad, 4 to 10 percent proteins, 1 to 2 percent lactose, and 72 to 76 percent whey solubles. It is not indicated whether the milk samples came from the triazole or phenyl ^{14}C-labeled portion of the study. The samples are merely described as being from days 2, 3, and 4.

The HPLCs of the soluble whey fractions show 4 to 5 peaks. The day 2 and 3 samples were dominated by the glycol RH-294 (see table below). The only other identified component was the alcohol RH-9090 (7 to 8% of day 2-3 samples). Four other more polar compounds were observed, but not identified. The relative amounts of these components are summarized in the following table.

Peak	Percent of Whey Activity Day 2	Day 3	Day 4
Polar peak A	--	--	42.4
Polar peak B	14.9	10.2	18.1
Polar peak C	7.1	4.5	3.4
Polar peak D	--	17.1	14.9
RH-294	71.0	60.6	21.3
RH-9090	6.8	7.8	--

We question whether the compounds designated B and C are the same in all cases. For example, the retention times for C vary from 10 to 12 minutes in the five chromatograms. Peak B is close to 9 minutes in most cases but drops to 8 minutes in the day 4 sample. These shifts are not observed for peak D, RH-294, and RH-9090, which show consistent retention times.

Taking into account that the soluble whey contained 72-76 percent of the milk activity, RH-294 comprised about 45-50% of the milk residue on days 2 and 3 and RH-9090 about 5%. Three or more unidentified polar compounds each represented about 3 to 11 percent of the residue. In the day 4 sample there was a marked shift to unidentified polar compounds including the new peak A at 32% of total milk activity, 14% B, 3% C, and 11% D. The glycol RH-294 comprised only 16% of the day 4 milk residue.

The petitioner notes that another ^{14}C study involving dosing of cows with a mixture of parent fungicide, RH-9090, and RH-9089 also found four polar metabolites in milk. Three of these co-chromatographed with urine metabolites which are claimed to be conjugates of RH-9090 and RH-9089. This additional study (Report #31H-86-18) was submitted in PP#7F3476 and will be reviewed later in conjunction with those permanent tolerance requests. (One comment we have at this time is that RH-9089 does not have a site amenable to conjugation.)

No characterization of residues in animal tissues or eggs has been submitted in conjunction with temporary tolerance petitions. Taking into account the low total activities observed in feeding studies (see Meat, Milk, Poultry and Eggs) and the limited experimental use of myclobutanil, we are willing to overlook this absence of data and accept regulation of only the parent compound in animal commodities for temporary tolerances. Method 310-84-13 can measure RH-3866 in meat, milk and eggs (see Analytical Methods below). Since we will be regulating different residues in plants and animals, a revised Section F is required which lists animal tolerances under a separate heading specifying residues of myclobutanil only. The crop tolerances will remain under the proposed expression including metabolites.

The residues to be regulated and measured under permanent tolerances will be determined after our review of additional metabolism studies in PP#7F3476. These include a poultry study (31H-86-17) and another dairy cow study in which dosing was done with a mixture of parent, RH-9090, and RH-9089 (31H-86-18). RH-294 is likely to be regulated as a major residue in milk. Also, RH-9090 will probably be a residue of concern in poultry as it is stated to be a major metabolite in tissues and eggs (Summary and Discussion Volume of PP#7F3476). The hydroxylactone may also have to be determined by the method for animal commodities.

Additional ^{14}C feeding studies in which residues were not identified (but merely quantitated as total activity) are described below under Meat, Milk, Poultry, and Eggs.

Analytical Methods

The petitioner has described methods to measure (1) the parent compound (RH-3866) in crops (Technical Report # 310-83-23); (2) parent plus free RH-9090 in crops and parent in animal commodities (TR # 310-84-13); and (3) parent, RH-9089 (after reduction to RH-9090), free RH-9090, and conjugated RH-9090 in crops (TR # 310-84-27).

Our January 9, 1985 review of PP#4G3149 discussed the first two methods noted above. These involve methanol extraction, cleanup by partitionings and Florisil column chromatography, and gas chromatography. Validation data were provided for RH-3866 in crops, meat, milk and eggs down to 0.01 ppm. Recovery data for RH-9090 involved crops only. See our 1/9/85 memo for details on actual recovery values.

The current petition includes an Addendum dated February 12, 1986 for Method 310-84-13 (EPA Accession No. 266030 for the Confidential version; none given for "clean" copy). The prescribed change is to dissolve the final residue in 3% methanol in toluene (instead of pure toluene) for GC analysis. The addition of methanol prevents adsorption of the analytes onto glass surfaces.

At this time we conclude that Method 310-84-13 can serve as the enforcement method for temporary tolerances for residues of RH-3866 in animal commodities. As noted above under Nature of Residue, it is likely that additional residues such as RH-9090, the hydroxylactone, and RH-294 will be regulated in meat, milk, and eggs for permanent tolerances. If that is the case, analytical methods with recovery data will be required for these compounds.

The method which measures RH-3866, RH-9089, and RH-9090 (free plus conjugated) in fruit is entitled "RH-3866 Total Residue Analytical Method for Apple and Grape" (Technical Report #310-84-27). The method is dated Ocober 16, 1984 and was submitted previously in PP#4G3149 (EPA Accession No. 073600), but not reviewed. "Clean" (nonconfidential) copies of the method plus an Addendum have been provided in the present petition. A confidential copy of the Addendum is also included (EPA Accession No. 266030). The basic steps of the method are acidic soxhlet extraction, sodium borohydride reduction, assorted partitionings, Chelex 100-Fe^{+3} column chromatography, methylene chloride partitioning, Florisil column chromatography, and gas liquid chromatography.

Method 310-84-27 starts with chopping the fruit with dry ice and placing the ground sample in a soxhlet apparatus for overnight extraction with 0.5N HCl/methanol (16-hour reflux). The acidic reflux cleaves RH-9090 conjugates, producing the free alcohol. After cooling and basification, $NaBH_4$ is added for 20 minutes to reduce the ketone RH-9089 to the alcohol RH-9090. Aqueous NaCl (2%) is added and the resulting aqueous methanol solution washed with pet ether before extracting the analytes into methylene chloride. After evaporation of the solvent at reduced pressure the residue is dissolved in 1:4 methanol-water and placed on an iron-activated Chelex 100 column. The analytes bind tightly to the Fe^{+3} on the column and are eluted by addition of a strong ligand (0.5N triazole in 1:1 methanol-water). After addition of sodium chloride, the analytes are extracted into methylene chloride and the latter washed with water to remove traces of triazole. After removal of the methylene chloride, the residue is dissolved in toluene and placed on a 5 percent water deactivated Florisil column. Myclobutanil is eluted with 1 percent methanol in toluene. The column is then washed with 3 percent methanol in toluene before eluting the metabolite RH-9090 with 7.5 percent methanol/toluene. After removal of the solvents at reduced pressure, RH-3866 and RH-9090 are dissolved in 1 percent methanol/toluene and 5 percent methanol/toluene, respectively, for gas chromatographic analyses (2% OV-101 column). The specified detectors are nitrogen/phosphorous and electron capture for parent and metabolite, respectively.

The modifications noted in the Addendum (July 8, 1986; Lab Project ID 31H-86-15) are to improve sample cleanup and quantitation of RH-9090. These include preparation of the Chelex Fe^{+3} column <u>fresh daily</u> using 0.5% ferric chloride solution (versus saturated), washing the methylene chloride layer <u>three</u> times with water to remove triazole, and substitution of Bio-Sil A for Florisil (acetone-toluene solvents as eluents). The GLC conditions (column, flow rate, temperature) are also changed for RH-9090.

Peak areas on the chromatograms are compared to standards of RH-3866 or RH-9090 with residues then reported as the sum of RH-3866 and "total RH-9090." The latter refers to the fact that the RH-9090 peak represents the free plus conjugated alcohol as well as the ketone RH-9089, the latter having been reduced by sodium borohydride.

Confirmatory analyses involve switching the detectors for the two analytes and also modifying the column temperatures. An additional alternative is use of a different column (2% OV-17 + 1% OV-210).

Untreated samples (controls) generally give no measurable peaks or small ones equivalent to < 0.01 ppm RH-3866 or RH-9090. Occasional values of 0.01 to 0.02 ppm are observed for controls. The only control we located over 0.02 ppm was 0.097 ppm RH-3866 in untreated dry apple pomace.

In the original method report, recoveries of RH-3866 and RH-9090 are reported as "92.1 ± 17.2%" and "83 ± 20.4%," respectively. The number of fortifications and the individual recovery values are not given. However, by examining the residue studies in the same volume (EPA Accession No. 073600) we have obtained actual recoveries. For 23 fortifications of parent compound RH-3866 in apples and grapes at levels of 0.01 to 2 ppm, recoveries averaged 92 percent (56 to 131% range; 3 values < 70%). For the metabolite RH-9090 recoveries ranged from 59 to 118 percent (85% average; 6 values < 70%) for 25 spikes (0.1 to 1.0 ppm).

The efficiency of the extraction step itself was shown by soxhlet extraction of radiolabeled grapes and apples (field treated). The total activities of the fruits (measured by combustion) were compared to those of the extracts. The extraction efficiencies were 95 to 96 percent. The samples were not analyzed further to determine what percentage of the total activity is actually quantitated by the method as RH-3866 plus RH-9090.

We note that some chromatograms were included in the original method report for control, treated, and fortified samples. The reported recoveries and ppm cannot be verified due to the absence of a calibration curve or responses for standards. Qualitatively, the chromatograms appear acceptable, although one apple sample and the confirmatory grape chromatograms have the analyte peak on the steep side of the solvent front.

The chromatograms obtained when the modifications listed in the Addendum are employed show the analyte peaks further from the solvent peak. Recoveries of 69 to 88 percent are reported on these chromatograms for four spiked samples (two with RH-3866 [0.1, 0.8 ppm], two with RH-9090 [0.05, 0.17 ppm]). We can verify at least three of these using the raw data provided.

Additional recovery values using the modified method are available in the residue data for apples, grapes, and processed commodities (discussed later in this review) (EPA Accession Nos. 266031 and 266032). These are summarized in the following table.

Substrate	Analyte	Ppm added	Recoveries
Fresh fruit	RH-3866	0.12-0.37	60-119 (89% avg) (n = 5)
Byproducts*	RH-3866	0.04-0.50	46-138 (84% avg) (n = 17)
Fresh fruit	RH-9090	0.025-0.43	55-109 (75% avg) (n = 5)
Byproducts*	RH-9090	0.025-0.38	42-91 (70% avg) (n = 12)

*Juices, pomaces; grape wine, stems, raisins.

The recoveries obtained with the modified method are thus somewhat lower than those from the original procedure (averages of 92% for RH-3866 and 85% for RH-9090 in fresh fruit).

Overall we conclude that an adequate analytical method is available to enforce the temporary tolerances for residues of RH-3866 and its metabolites in apples, grapes, and their byproducts.

For permanent tolerances the following additional data will be required for the analytical method for plants:

1. Recovery data for RH-9090 glucoside in apples and grapes at levels down to at least 0.05 ppm. Several other fortifications in the range of 0.1 to 1.0 ppm should also be tested. At this time we consider the method for free RH-9090 at best only marginally acceptable (8 of 17 spiked samples in processing studies had < 70% recovery). The results with the glucoside will determine whether the method needs to be modified to improve recoveries of RH-9090.

2. We note that a method for the ketone RH-9089 in plants will not be required for permanent tolerances. Therefore, the petitioner need not generate validation data for that metabolite. It is his option as to whether the borohydride reduction be kept in the analytical method for plants. A final decision on what animal metabolites must be determined by analytical methods awaits our review of additional metabolism studies in PP#7F3476.

3. Method trials will be requested in the near future for both the Total Method (310-84-27) on grapes and apples

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and Method 310-84-13 on animal commodities. Completion of these trials will not be necessary prior to approval of temporary tolerances.

Residue Data

Storage Stability

Interim reports on the storage stability of RH-3866 in apples and grapes were submitted in an amendment to PP#4G3149, but have not been reviewed (EPA Accession No. 073600; Technical Report Numbers 310-85-03 and 310-85-02). In these experiments untreated apples or grapes were chopped with dry ice and the latter allowed to sublime in a freezer. Four 10 gram samples were then placed in glass bottles for each interval to be examined. The four samples represented a control, a control to be fortified at analysis time for method validation, and two samples to be fortified for the storage stability measurements. The latter received 1 ug RH-3866 (therefore, 0.1 ppm) in 1 mL toluene and were dried in a hood for 4 hours before going into a freezer (-15 °C). Zero-day samples, including one fortified with 1.3 ppm ^{14}C-RH-3866 to verify the method's extraction efficiency, were analyzed immediately. Other samples were examined after 7, 14, 28, 90, and 194 days of storage. Method 310-84-13 was used to measure residues of myclobutanil.

All control samples are reported as "no detectable residue." We estimate 0.002 to 0.008 ppm RH-3866 equivalents in these samples based on the small peaks (1-3 mm) present in some chromatograms. Recoveries of fresh spikes to validate the method were 89 to 104 percent for apples and 92 to 106 percent for grapes. Extractability of the ^{14}C material was 99+ percent. Recoveries of stored fortifications were also excellent: 89 to 107 percent apples, 93 to 108 percent grapes. The submitted chromatograms support the reported results.

The 6-month results of the grape and apple studies agree with the 1-year wheat study (January 9, 1985 review, PP#4G3149). Residues of RH-3866 in these crops are stable during frozen storage for up to 1 year. (We note that the Summary in PP#7F3476 indicates the apple/grape studies have now been carried out for 2 years with no significant loss of myclobutanil. The completion of these studies will be reviewed in connection with that permanent tolerance petition.)

Fresh Fruit

Residue data for grapes and apples were submitted in the May 23, 1985 amendment to PP#4G3149 (Accession Nos. 073599 and 073600). These included residue decline studies in which radiolabeled RH-3866 was applied to grapes and apples. Fruit residues as a function of time were so scattered that half-life calculations could not be completed. These decline studies will not be discussed further as they provided little useful information.

Field trials for grapes were conducted in California (7 sites) and New York (1 site) in 1984 using the 2E formulation of myclobutanil. Three sites in CA involved treatment with only 1 oz ai/A (half the proposed dosage). Total residues of RH-3866 plus its metabolites were 0.012 to 0.046 ppm with 14-day preharvest intervals (PHIs). The other four CA locations plus the NY trial examined rates of 2.0 to 2.13 oz ai/A (1.0-1.06X proposed rate) with total seasonal dosages of 0.63 to 0.88 lb ai (versus 0.6 and 0.9 lb ai proposed for 40W and 60DF formulations). Combined residues of RH-3866 plus "total RH-9090" were 0.052 to 0.224 ppm with 13- to 15-day PHIs (14 days proposed). Residues of RH-3866 and RH-9090 were corrected for average recoveries of 90 percent and 70 percent, respectively.

With the above data we can conclude that the established temporary tolerance of 0.5 ppm for myclobutanil plus its metabolites on fresh grapes is adequate. Additional data in PP#7F3476 show residues greater than 0.5 ppm in a few cases (Table 7 in Summary and Discussion Volume). However, it is noted these were "atypical applications by hand-sprayer" in "trials not representative of EUP trials with commercial equipment." Since the experimental use permit labels specify airblast or hydraulic equipment, we will not consider the values > 0.5 ppm applicable for setting temporary tolerances. They will be considered relevant for permanent tolerances. Rohm & Haas apparently has already done this since a 1 ppm tolerance is proposed for grapes in PP#7F3476.

For a permanent tolerance on grapes, additional trials reflecting the maximum application rate (per spray and per season) for both the 40W and 60DF formulations should be submitted. Both CA and NY sites should be included in these studies. Data reflecting aerial sprays are needed if this mode of application is desired to be included on the label.

Field trials for apples were carried out in Wisconsin, Pennsylvania (2 sites), Washington, Virginia, and South Carolina on assorted varieties such as McIntosh, Rome Beauty, and Jonathan. The wettable powder formulation was applied at rates ranging from 0.09 to 0.5 lb ai/A (versus 0.25 lb proposed). At each location at least one set of samples received close to or more than the maximum seasonal dose of 2 lb active ingredient on the 40W label. (The 60DF label calls for up to 3 lb ai per season, but we have questioned this discrepancy.) PHIs were scattered - 6 days in SC, 12 to 14 days in PA and WI, 23 days in VA, and 129 days in

WA. The maximum residue observed in samples reflecting close to the proposed use was 0.331 ppm RH-3866 plus total RH-9090. One sample (SC) contained a higher residue (0.437 ppm) following applications of only 0.56X (0.14 lb ai/A). Whether this indicates the 0.5 ppm tolerance could be exceeded is difficult to determine since the sample had a short PHI (6 days versus 14 days proposed). At this time we conclude the established 0.5 ppm temporary tolerance is adequate. However, for permanent tolerances additional studies in major apple production areas should be submitted. Application rates and schedules should reflect proposed labels as closely as possible.

Processed Commodities

A grape processing study has been submitted in the current petition (EPA Accession No. 266032; Technical Report No. 31H-86-11). Two samples of grapes, both of which received 5 x 0.1 lb/ai RH-3866 per acre with 14-day PHIs, were processed at California State University (Fresno) into juice, pomaces, stems, and wine. Raisins were also produced in one instance. The total residues in the assorted fractions are summarized below, corrected for recoveries of 90 percent (RH-3866) or 70 percent (RH-9090).

Sample	Total Ppm	Conc. Factor
Fruit (85-0419)	0.598	--
Juice	0.072	0.12
Wet pomace	0.807	1.35
Dry pomace	1.261	2.11
Wine	0.132	0.22
Stems	1.523	2.55
Fruit (85-0323)	0.171	--
Juice	0.059	0.35
Wet pomace	0.091	0.53
Dry pomace	0.997	5.83
Wine	0.061	0.36
Stem	NDR	--
A + B raisins	0.940	5.50
C raisin	1.07	6.26
Midget raisin	0.932	5.45
Waste raisin	4.227	24.7

From the above it can be seen that residues in juice and wine are considerably less than those in fruit, while significant concentration occurs in dry pomace and raisins. The petitioner has concluded that the concentration factors to use for determining food/feed additive tolerances are 2X for wet pomace, 6X for dry pomace, 7X for raisins, and 25X for raisin waste. We can concur with these figures. Therefore, we consider the proposed tolerances of 5 ppm for residues of myclobutanil and its metabolites in dry grape pomace and raisins reasonable. However, the current practice is to set one tolerance for "pomace" only. Therefore, "dry" should be deleted as well as the 1 ppm tolerance for wet grape pomace. Also, the 12.5 ppm feed additive tolerance for raisin waste should be changed to 15 ppm since fractional tolerances > 1 ppm are generally avoided.

We note that the one sample of fresh grapes contained residues above the 0.5 ppm temporary tolerance. These grapes were treated with a hand sprayer which, as noted by Rohm & Haas, "can result in direct deposition of a larger percentage of the spray formulation directly onto the fruit causing higher residues than mist blower application." As stated earlier in this review, this must be taken into account when establishing permanent tolerances.

An apple processing study has also been included in this petition (Technical Report #31H-86-09; EPA Accession No. 266031). Golden Delicious apples grown in Ohio received 14 applications of 0.25 lb ai/A during the summer of 1985. The fruit were harvested 21 days after the final application and processed at Rohm & Haas into cider (juice) and pomaces. The apples were quartered, ground, and placed in a nylon bag in a wine press for 30 minutes to yield juice and wet pomace. A portion of the latter was dried in a hood to produce dry pomace. Analyses for RH-3866 and its metabolites (RH-9090 and its glucoside; RH-9089) were then conducted using Method 310-84-27 with its Addendum. As in field trials, residues of parent and metabolites were corrected for recoveries of 90 percent and 70 percent, respectively. Total residues in fruit, juice, wet pomace, and dry pomace were 0.233, 0.038, 0.478, and 1.578 ppm, respectively. Thus, as was the case with grapes, residues concentrated in dry pomace, but were much lower in juice. The concentration factors for wet and dry apple pomace are 2.05 and 6.77 ppm, respectively. Therefore, the proposed 5 ppm feed additive tolerance for dry apple pomace is adequate. However, as noted above for grapes, the term "dry" should be dropped from the tolerance and the wet pomace tolerance deleted.

Although details and raw data have not been supplied in the current petition, the Summary and Discussion Volume (EPA Accession No. 266105) for PP#7F3476 describes the results of processing a second batch of apples. Total residues of RH-3866 plus metabolites in fruit, cider, wet pomace, and dry pomace are reported to be 0.050, 0.019, 0.092, and 0.306 ppm, respectively. The concentration factors are therefore 1.84 and 6.12 for wet and dry pomace. Since these values are very close to those observed in the above apple study, we will not require submission of additional details on this second experiment.

Meat, Milk, Poultry, and Eggs

In order to determine the extent to which myclobutanil transfers to animal tissues, milk, and eggs, Rohm & Haas has conducted multilevel dairy cow and poultry feeding studies in which radiolabeled material was administered. For comparison purposes in cattle, we also have the ^{14}C study (one cow with phenyl label and one with triazole label) discussed earlier in this review and in our January 9, 1985 review of PP#4G3149.

The multilevel cow-feeding study has been submitted twice. In PP#4G3149 it was entitled "^{14}C-RH-3866 Dairy Cow Residue Metabolism and Feeding Study" (EPA Accession No. 073599). The report was designated "ABC Laboratory Number 31726," but most of the appendices were not included. The current petition includes the same report with all the appendices plus an "Executive Summary" prepared by Rohm & Haas (EPA Accession No. 266028).

The study was conducted according to Rohm & Haas protocol #310-84-02. The in-life portion was carried out at the University of Missouri Sinclair Research Farm with the subsequent radioanalyses performed by Analytical Bio-Chemistry Laboratories, Inc. (ABC Labs).

Jersey cows were placed in individual stalls in an insulated barn for a 12- to 13-day acclimation period. Dosing was then initiated with a 32:58:10 mixture of ^{14}C RH-3866/RH-9090/RH-9089. The parent compound was labeled in the phenyl ring, whereas the metabolites carried a triazole label. The radioactive mixture was placed on ground wheat in gelatin capsules, which were administered by a balling gun after each a.m. milking. The intended dosage levels were 0.915, 3.05, 9.15, and 30.5 ppm. The actual doses based on feed consumption and radioanalysis of capsules were 1.18, 2.83, 11.8, and 38.3 ppm. Two cows were employed at each dose level with a ninth animal serving as a control. Dosing was continued for 10 consecutive days.

Each cow received 6 kg dairy ration plus 9 kg alfalfa cubes daily plus a mineral supplement. Water was available ad libitum. Refused food was weighed each morning to determine actual consumption. Both a.m. and p.m. milkings were conducted by machine with samples collected on the day before the initial dose up through sacrifice. Although average milk production dropped in all cows for the acclimation period versus previous production on the farm, in most cases it increased slightly during the dosing period. No drastic weight changes were observed from the start of acclimation until sacrifice.

The cows were sacrificed by stun gun about 24 hours after the last dose. The organs sampled for analysis included liver, kidney, heart, muscles (semimembranous, triceps, longissimus dorsi), and fats (omental, perirenal, mediastinal).

Tissues were ground before subsampling for radioanalysis. Nonfatty tissues were combusted, while fats were sonicated with Carbosorb to solubilize them before adding Permafluor. Milk was mixed directly with ionized water and scintillation fluid. Assorted tissues were spiked with ^{14}C-benzoic acid at levels of 0.1 to 2.0 ppm to determine the efficiency of the radioassay. Recoveries were 90 to 105 percent.

Radioactivity in milk plateaued by day 2 or 3 at all feeding levels. The maximum values in the ABC Report summary table were averages within each dosage level. Our concern lies in the residues in each sample. Inspecting the raw data tables we find that the maximum residues in individual samples of milk were 0.009, 0.024, 0.074, and 0.198 ppm for the four dosages. According to the Rohm & Haas Executive Summary these "ppm" values refer to "RH-3866 equivalents." [Since the molecular weights of the three dosing compounds are close (288.8, 304.8, 305.8), the ppm in terms of the original dose would not be significantly different.] Reported residues are consistent with the raw data (cpm, counting efficiencies, specific activity, sample weight).

In the case of tissues the ABC Report again summarized the mean values for each group. We have scanned the raw data tables to obtain the ranges of residues observed for individual samples. These are listed in the table below. We note that the Rohm & Hass Executive Summary lists some maximum residues which we were not able to locate (specifically, 0.098 ppm for the muscle and 0.073 ppm for fat in the highest dose level). The residues again are in terms of RH-3866 equivalents.

Ppm Residue

	Doses			
Tissue	1.18 ppm	2.83 ppm	11.8 ppm	38.3 ppm
Muscles	< 0.02	< 0.02	< 0.02	0.013-0.042
Fats	< 0.02	< 0.02	< 0.02	0.018-0.028
Kidney	< 0.02	< 0.02	0.038-0.066	0.121-0.188
Liver	0.044-0.048	0.100-0.108	0.272-0.322	0.669-0.974

The results of the above multilevel study using a mixture of myclobutanil and metabolites as the dose agree with those obtained by feeding two cows ^{14}C-RH-3866 only. This study was discussed in the January 9, 1985 R. Loranger review of PP#4G3149 and earlier in this memorandum (see Nature of Residue). Following administration of 10 ppm myclobutanil in the diet for 10 days the maximum total activities in tissues were 0.014 ppm for muscle, 0.036 ppm for fat, 0.11 ppm for kidney, and 0.42 ppm for liver. These values are somewhat higher (but less than 2X) than those found in the 11.8 ppm level of the above study. Comparing milk residues in the two studies, we again find they were similar. However, unlike tissues, the maximum in the previous study (0.043 ppm) was lower than that observed in the 11.8 ppm dosing level of the multilevel study (maximum 0.074 ppm total activity).

The cattle feed items in this EUP are apple pomace, grape pomace, and raisin waste. We estimate a maximum dietary burden of 2.75 ppm for beef cattle based on 10 percent raisin waste (using 12.5 ppm residue on this feed for calculation purposes) and 30 percent dry grape pomace (5 ppm tolerance). Dairy cattle could consume about 2.25 ppm RH-3866 plus its metabolites (20% dry grape pomace plus 10% raisin waste). We consider it highly unlikely that apple pomace and grape pomace would be fed together (at least at the maximum percentages of the diet shown in the Residue Chemistry Guidelines). Using the maximum total activities observed in the cow ^{14}C feeding studies we calculate that these dietary burdens will result in the following maximum residues: 0.12 ppm, liver; 0.03 ppm, kidney; 0.002 pm, fat; 0.003 ppm, muscle; and 0.014 ppm, milk.

The petitioner has not explained how he arrived at the proposed tolerances. We consider the proposed 0.5 ppm for liver and 0.1 ppm for milk too high based on the above calculations. They should be decreased to 0.2 and 0.02 ppm, respectively. The 0.04 ppm for meat and meat byproducts is reasonable. However, fat should be added to this tolerance as well as it being specified to apply to cattle, goats, hogs, horses, and sheep.

Generally we prefer that feeding studies involve measurement of residues with the "cold" analytical method that determines the toxic residue of concern. However, in the present cases for cattle and poultry (study details below), total residues based on radioactivity are so low that we can accept these ^{14}C studies as the "conventional" feeding studies. If the studies were to be repeated using cold material, detectable residues are likely to be found in only beef liver and perhaps kidney at the dietary burdens anticipated from the grape and apple uses. The petitioner should be warned, however, that future uses of myclobutanil which significantly increase dietary burdens for livestock may trigger the need for conventional feeding studies.

The poultry feeding study (EPA Accession No. 266029; Technical Report No. 31H-86-16) was also conducted using a radiolabeled mixture of RH-3866 and metabolites. Although the summary was prepared by Rohm & Haas, the actual work was carried out by the Agrisearch, Incorporated.

White Leghorn laying hens, each one in a layer cage, were acclimated 7 days prior to initiation of dosing with a 45:45:10 mixture of RH-3866/RH-9090/RH-9089. The parent compound was labeled with ^{14}C throughout the phenyl ring, while the two metabolites were labeled in the triazole ring. The dose was given along with layer mash in a gelatin capsule. The intended levels in the diet were 1, 3, 10, and 30 ppm. The actual doses based on radioassay were 1.05, 3.57, 10.2, and 28.3 ppm. Ten birds were used at each of the four levels (plus controls) with dosing continued for 28 consecutive days. (Two additional groups of three hens received 110 ppm ^{14}C-parent or metabolites for 7 days to provide tissues, eggs, and excreta for identification of metabolites. This report will be provided later as an addendum. It appears that it has been submitted in PP#7F3476 as Report 31H-86-17.)

The hens each received 110 g layer mash "that was completely consumed within a 24-hour period." Water was provided ad libitum. Eggs were collected each morning for production records. All eggs from each group were kept on days -1, 1, 2, 4, 7, 10, 14, 21, 28 through 32, 35, and 42, deshelled, and stored frozen. Two eggs from each level were separated into yolks and whites on days 1, 7, 14, and 28. Egg production and terminal body weights were similar for control and treated hens.

The sacrifice schedule was as follows: Four hens from each group were sacrificed less than 24 hours after the final dose, and three hens from each group were held 7 and 14 days after dosing prior to sacrifice to examine the effect of withdrawal on residues. The tissues collected after sacrifice included breast and thigh muscles, liver, kidney, gizzard, heart, and fat. We note that samples were pooled within each group.

Sample preparation for eggs entailed homogenization prior to combustion. Tissues were ground with dry ice and then combusted to measure total radioactivity. Quantitation limits varied from 0.002 to 0.045 ppm depending upon the substrate and feeding level.

Whole egg residues plateaued by day 7. The maximum residues observed for each feeding level appear in the table below. Yolk residues were somewhat higher than whites on a ppm basis. Egg residues declined upon removal of RH-3866 and its metabolites from the diet. After a 7-day withdrawal period all eggs had no detectable residues (< 0.002 to < 0.018 ppm depending on dose level/specific activity).

The following table summarizes total radioactive residues in eggs (maximum values) and tissues for all dosages. In all cases, the samples were pooled for each feeding level and then analyzed in duplicate. The "ppm" residues are in terms of RH-3866 equivalents.

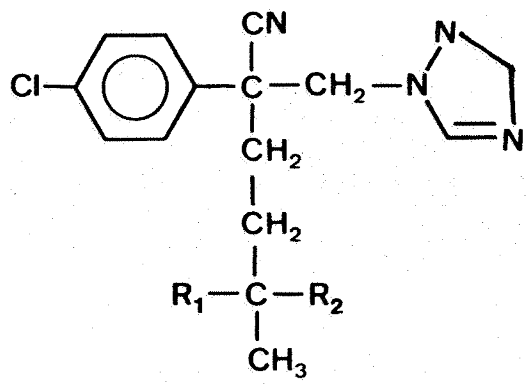
	^{14}C Residue (ppm) Feeding level			
Sample	1.05 ppm	3.57 ppm	10.2 ppm	28.3 ppm
Eggs	0.005	0.013	0.034	0.129
Breast muscle	<0.002	0.004	0.008	0.027
Thigh muscle	<0.002	0.003	0.006	0.019
Fat	<0.005	<0.005	<0.015	<0.045
Liver	0.003	0.006	0.018	0.047
Kidney	<0.002	0.003	0.006	0.021

If either dry apple pomace or dry grape pomace were to comprise 5 percent of the poultry diet, the intake of RH-3866 plus its metabolites would be 0.25 ppm. From the above table it can be seen that ingestion of this level would result in extremely low residues in eggs and tissues ($\leq$1 ppb = 0.001 ppm). Therefore, we consider the proposed 0.4 ppm tolerance for eggs too high. Since the analytical method (310-84-13) for RH-3866 in animal commodities was validated at 0.01 ppm, we suggest a tolerance of 0.01 ppm for eggs. A tolerance of 0.01 ppm should also be proposed for the meat, fat and meat byproducts of poultry. As noted under Nature of Residue, the animal tolerances should be listed under a separate heading specifying residues of myclobutanil only.

Attachment (Copy to all files)

cc: Circu, RF, PP#7G3479, Loranger, PMSD/ISB, M. Nelson, TOX
RDI: A.R. Rathman:5/27/87:R.D. Schmitt:5/28/87

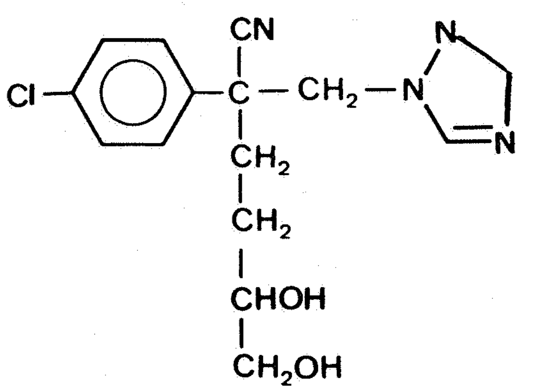
92009:I:Loranger:KENCO-15:KENCO:06/02/87:de:vo:de
Edited by R. Loranger



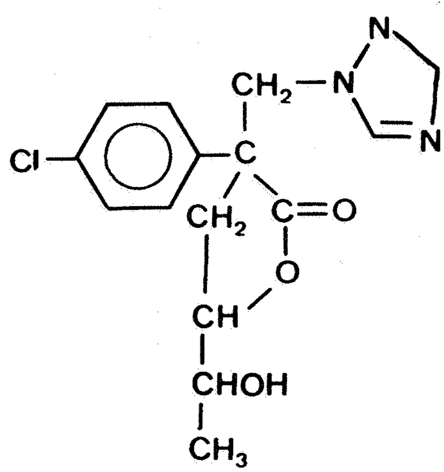
$R_1 = R_2 = H$ RH-3866; myclobutanil; α-butyl-α-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile

$R_1 = H$ $R_2 = OH$ RH-9090; α-(3-hydroxybutyl)-α-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile

$R_1 + R_2 = O$ (ketone) RH-9089



RH-294



Hydroxylactone

STRUCTURES OF RH-3866 AND ITS METABOLITES
ATTACHMENT TO PP#7G3479